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RESEARCH PAPER

Response of *Clarias gariepinus* (juveniles) Exposed to Sub-lethal Concentrations of Atrazine

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Abstract

This research was conducted to evaluate the toxicity effects of atrazine on *Clarias gariepinus* juveniles. *C. gariepinus* were purchased from Fish Farm in Akure and acclimated prior to toxicity tests for seven days in concrete tanks. The fishes were fed with commercial feed twice daily and stopped 24hrs to the commencement of the toxicity test. Different concentrations of atrazine were prepared (0.00, 0.10, 0.15, 0.20, 0.25 and 0.30 ml/L) in triplicate. Fifteen (15) *C. gariepinus* juveniles (20±0.6 g and 15.15±0.5 cm) were randomly distributed into each glass tank (60 x 30 x 40 cm^3) containing ten litres of treatment concentrations. Mortality, water parameters (DO2, pH, Temp and Conductivity) etc. were monitored at regular time interval (24, 48, 72 and 96 hours). Also, enzymes activities (Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Lactate Dehydrogense (LDH)) in fish exposed to atrazine were also measured

There was fluctuation in the values of water parameters with abnormal behaviours increased with increasing concentration of atrazine across the treatment groups. The LC50 was 0.183ml/l for 96 hours exposure period. There were significant ($P<0.05$) differences in AST, LDH, and ALT activities in the fish exposed to increasing atrazine across the treatment relative to control.

Introduction

The use of herbicides to control weeds has been recognized as a part of agricultural practices throughout the world. Unfortunately, the indiscriminate use of these herbicides to improve agricultural production and yield may have impacts on non-target organisms, especially aquatic life forms and their environment (WHO, 1994).

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is one of the most commonly used herbicides found in the rural environments (Battaglin, Rice, Focazio, Salmons & Barry 2009). It is extensively used on corn, sorghum, sugarcane, pineapples and to some extent on landscape vegetation. According to (Battaglin, Rice, Foazio, Salmons & Barry, 2009), mode of action of atrazine involves blocking of electron transport in photo system thereby leading to chlorophyll destruction and blocking of photosynthesis. When atrazine was first released for agricultural use, it was thought that since photosynthesis is limited to plants, animals would be immune to its effects (Ventura *et al*. 2008). The indiscriminate use of this herbicide, careless handling, accidental spillage or discharge of untreated effluents into natural water ways have harmful effects on the fish populations and other aquatic organisms and may contribute to long term effects in the environment (Ventura *et al*. 2008).

Atrazine is moderately toxic to aquatic animals, Battaglin *et al*. (2005), mobile in the environment and is among the most detected herbicide in streams, rivers, ponds, reservoirs and ground waters. It has a hydrolysis half-life of 30 days and relatively high water solubility (32 $mg{\cdot}L^{-1}$), which aids in its infiltration into ground water (Orme & Kegley, 2004). Due to the low

persistence of atrazine herbicide, repeated applications are practiced for the control of weeds in agricultural fields and as a result, large quantities of the herbicide find their ways into water bodies Neškovic, Elezovic, Karan, Poleksic, & Budimir (1993) reported that fish can serve as bio-indicators of environmental pollution and can play significant roles in assessing potential risk associated with contamination in aquatic environment. This is possible since they are directly exposed to chemicals resulting from agricultural production via surface runoff or indirectly through food chain of the ecosystem (Lakra & Nagpure, 2009). Fish is endowed with defensive mechanisms to counteract the impact of reactive oxygen species (ROS) resulting from metabolism of various chemicals or xenobiotics (Risso, Mercuri, Quagliaro, Damante, & Ceriello, 2001). Oxidative stress develops when there is an imbalance between pro-oxidants and antioxidants ratio, leading to the generation of ROS (Lius, Zhang, Liu, & Huang 2006). Environmental contaminants such as herbicides, heavy metals and insecticides are known to modulate antioxidant defensive systems and to cause oxidative damage in aquatic organisms by ROS production (Monteiro, Almeida, Rantin, & Kalinin, 2006).

Several characteristics of *C. gariepinus* such as wide distribution in the freshwater environment, availability throughout the year, easy acclimation to laboratory conditions and commercial importance make this species an excellent test for toxicity and biochemical studies (Lakra & Nagpure, 2009). This study aimed at examine the effects of atrazine on biochemical change and behavioural disorders in *C. gariepinus*.

Materials and Method

Experiment Animal

A total number of 300 apparently healthy *C. gariepinus* juveniles of 20±0.6 g and 15.5±0.5 cm were purchased from Jacular Fish Farm Akure on April 2017, and transported to the Teaching and Research Farm of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Ondo State Nigeria. The fishes were acclimatized prior to toxicity tests for seven days in concrete tanks of 1 x 2 x 1 (m^3) dimension half filled with fresh water. During the acclimation period, the fishes were observed for disease condition and general wellness Water was changed every three days to prevent build of metabolic wastes and also to increase oxygen supply. They were fed with commercial feeds of 2mm pelleted size twice daily.

Experimental Procedure

Feeding stopped 24 hours to the commencement of the test and during exposure period that lasted for 96 hours. This was done because feeding increases the rate of respiration and excretory products, which may influence the toxicity of test solution. The study consisted of the control (no atrazine inclusion) and five treatments (18 plastic transparent buckets of 20 liters capacity) containing 10 litres of water were prepared in triplicate in static bio-assay in with different concentrations (0.10, 0.15, 0.20, 0. 25 and 0.30 ml/l) of atrazine after a range finding test has been performed. Thereafter, fifteen individual fish per concentration of the toxicant were exposed for 96 hours bio-assay test.

The behavioural activities and morphological responses of *C. gariepinus* juveniles exposed to different concentration of atrazine were observed physically. Control tank were also monitored along with the toxicant concentrations to provide a reference for assessing any behavioural or morphological changes on the test organisms. The number of dead and living fishes in each concentration were counted and removed after every 24 hours. The behavioural and morphological characteristics that were monitored include erratic and vertical swimming, loss of reflex, restlessness and colour change, mucus secretion, deformities and haemorrhage. The 96-hours LC50 toxicity for each atrazine concentration was determined as a summary of percentage mortality data following the method of Hoquel, Mirza, & Miah (1993).

Measuring Water Quality Parameters

The temperature, pH, dissolved oxygen and conductivity were monitored before, during and after the experiment using methods described by APHA (1985) using a digital Dual purpose meter (Model JENWAY 3150).

Biochemical Analysis

After 96 hours of the bio-assay, portion of blood were collected and labelled and later allowed to clot at room temperature for 30 minutes. The clotted samples were subjected to centrifugation at 4500 rpm for 15 minutes to separate the serum sample from both control and treated fish and were stored at -20°C. The aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogense (LDH) activities were determined according to Reitman & Frankel (1957), colometric method using Randox kits.

Data Analysis

Data collected from sub-lethal test were analysed using probit) analysis in SPSS software version 16 as described by Finney (1952). Data obtained from water physio-chemical parameters and enzymes activities were analysed by one factor analysis ANOVA where mean are significantly different, New Duncan Multiple Range Test were used for follow up analysis using MINITAB 14 software. To examine the effects of water parameters on the concentration that produce LC50, response surface methodology was used

Result

The behavioural responses of the fish exposed to atrazine was observed in different level of concentration as well as control (Table 1.) Normal swimming behaviour and natural colour were observed in the control throughout the exposure period while abnormal behaviours displayed by the fish increased with increasing concentration of the toxicant. The result of the physiochemical parameter of the water obtained before, during and after the experiment at regular time interval of 24, 48, 72 and 96 hours respectively are showed in Table 2. This study revealed the effects of atrazine on physico chemical parameters of the water during the experimental. The values of dissolved oxygen decrease with increase in concentration of the toxicant while that of conductivity increases with concentrations. Temperature also increases but no significant difference in the values obtained. The pH was noticed to increase compared with the control.

Figure 1 shown the probit output for LC50 for *C. gariepinus* exposed to atrazine concentrations. From the graph, the LC50 was 0.183ml/l. In order to ascertain the corresponding water parameters that produced LC50, the surface response methodology (SRM) was used. The surface graph represents a set of three-dimensional data (Atrazine concentration, mortality and water parameters). The results showed the corresponding values for water parameters (Temperature, Dissolved oxygen, pH and conductivity) measured in relation to the concentration of atrazine and mortality during 96 hours exposure of *C. gariepinus* to atrazine. Figure 2 shows the relationship between atrazine concentration, percentage mortality and temperature. The result revealed 27.17°C as the temperature at which LC50 was obtained. The relationship between dissolved oxygen, atrazine concentration and percentage mortality is presented in Figure 3. The result shown that at concentration of 0.183ml/l that produced LC50 of the test organism was achieved with corresponding value of 6.60 mg/l Also, in Figure 4 relationship between pH, concentration and mortality is compared and the value of pH was 7.15. The relationship between conductivity, concentration and mortality is presented in Figure 5. The result displays corresponding value for conductivity with LC50 in of *C. gariepinus* exposed to atrazine

The results of the enzymes activities in fish exposed to various concentrations of atrazine are presented in Table 3. The results showed that the aspartate aminotransferase (AST) increased with increasing level of the atrazine. However, there were significant $P<0.05$) differences in the values recorded across the treatments. There was significant difference ($P<0.05$) in values of lactate dehydrogenase (LDH) observed in the experiment with the values of lactate dehydrogenase (LDH) values increased with increasing level of the atrazine. The values of the alanine aminotransferase (ALT) also increased with increasing concentration of the atrazine at each successive level across the treatment.

Discussions

The African catfish *(Clarias gariepinus),* an ecological important and highly valued food fish in

Table 1. Behavioural changes and biological responses in *Clarias gariepinus* juvenile exposed to different concentrations of Atrazine

EXP /BEH	24 Hours						48 Hours						72 Hours						96 Hours					
CONC (ml)	0.0	0.10	0.15	0.20	0.25	0.30	0.00	0.10	0.15	0.20	0.25	0.30	0.00	0.10	0.15	0.20	0.25	0.03	0.00	0.10	0.15	0.20	0.25	0.30
A. G	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
E. S	-	-	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
L.R	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	+	+	+	-	-	+	+	+	+
C& R	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
M.S	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
D&H	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+

- = No change in behaviour found, + = change in behaviour found; %= Concentration mg/L; A.G =Air gulp, E.S =Erratic swimming, L.R = Loss of reflex, C&R= Colour change and Restlessness, M.S = Mucus secretion, S=, D&H= Deformities and Haemorrhage.

Table 2. Physiochemical parameters obtained during exposure of *Clarias gariepinus* juveniles to atrazine for 96 hours (Means ±SE)

Concentration (ml/L) Parameters	0.00 (control)	0.10	0.15	0.20	0.25	0.35
DO_2 (mg/l)	6.88±0.05^a	6.39±.0.01^b	6.36±0.00^b	6.27±0.03^c	6.16±01^d	5.99±0.02^e
pH	6.95±0.17^d	7.09 ±0.00^c	7.15 ±0.00^b	7.20±.00^a	7.24±0.00^a	7.19±0.09^a
Temp (^oC)	27.10±0.00^a	27.13±0.33^a	27.16±0.33^a	27.20±0.00^a	27.20±0.00^a	27.20±0.00^a
Conductivity (µS/cm)	76.5±0.12^f	83.23±0.54^e	85.27±0.05^d	91.14±0.38^c	97.66±0.32^b	99.08±0.09^a

Means in the same column with same superscript are not significantly different (P>0.05).

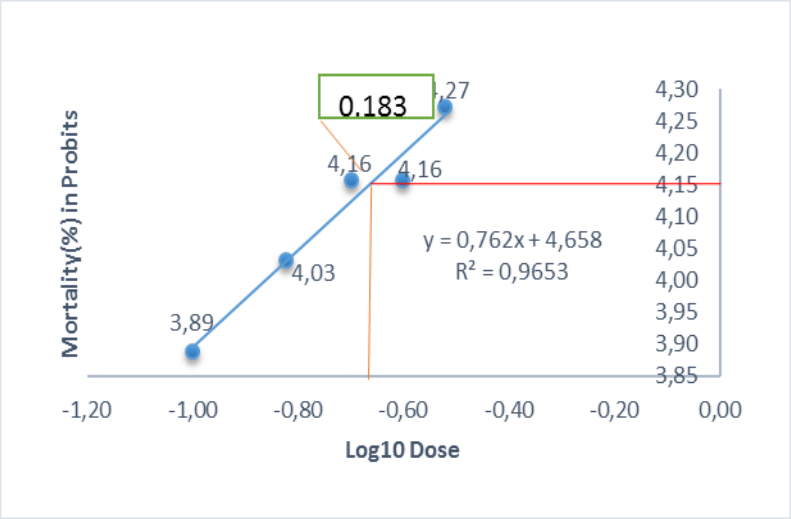

Figure 1. LC50 of *Clarias gariepinus* exposed to different concentrations of atrazine.

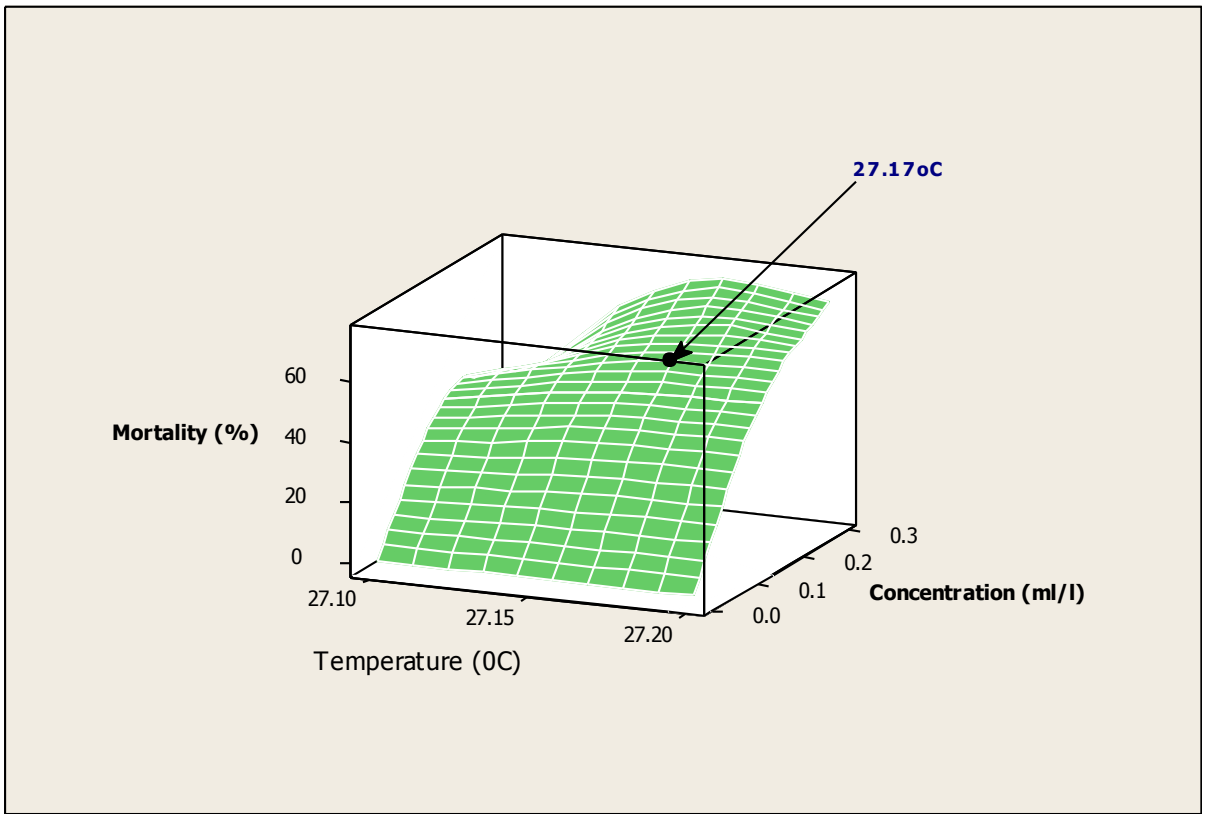

Figure 2. Corresponding temperature for LC50 for *C. gariepinus* juveniles exposed to various concentrations of atrazine.

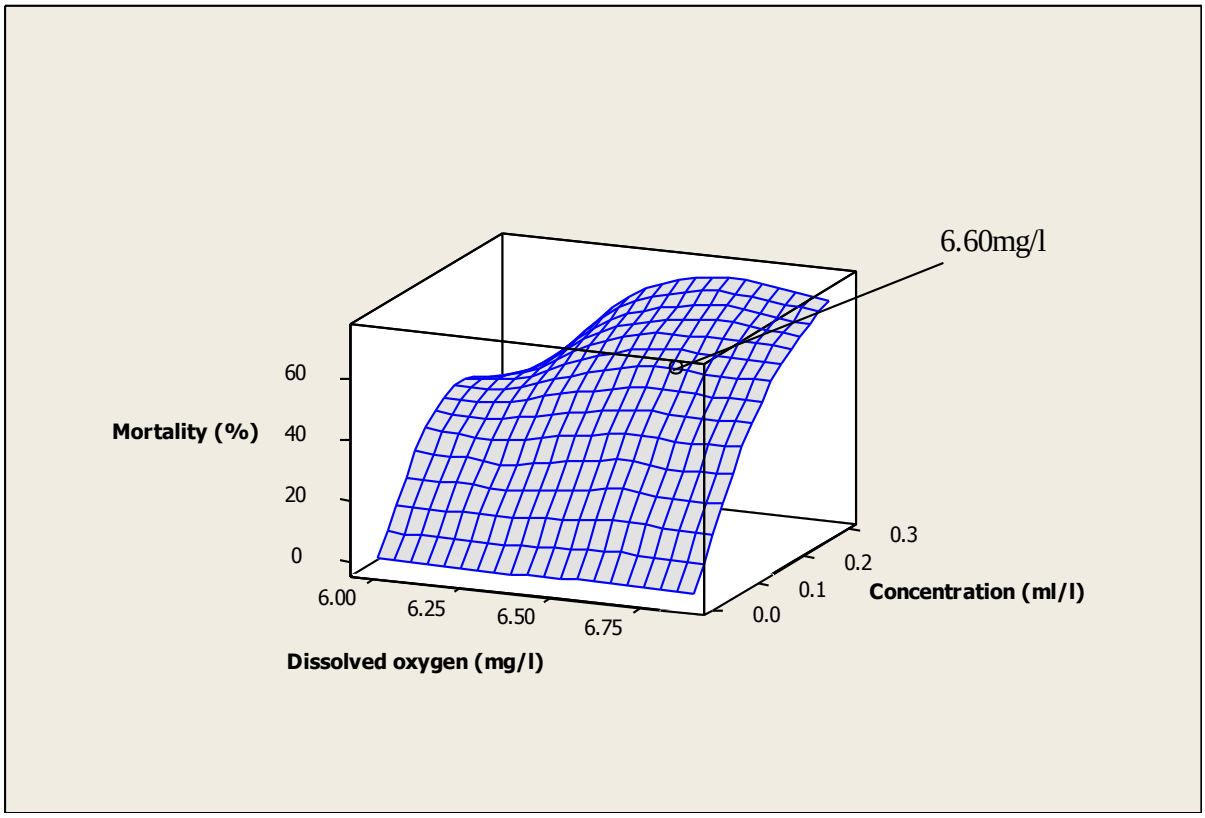

Figure 3. Corresponding dissolved oxygen for LC50 for *C. gariepinus* juveniles exposed to various concentrations of atrazine

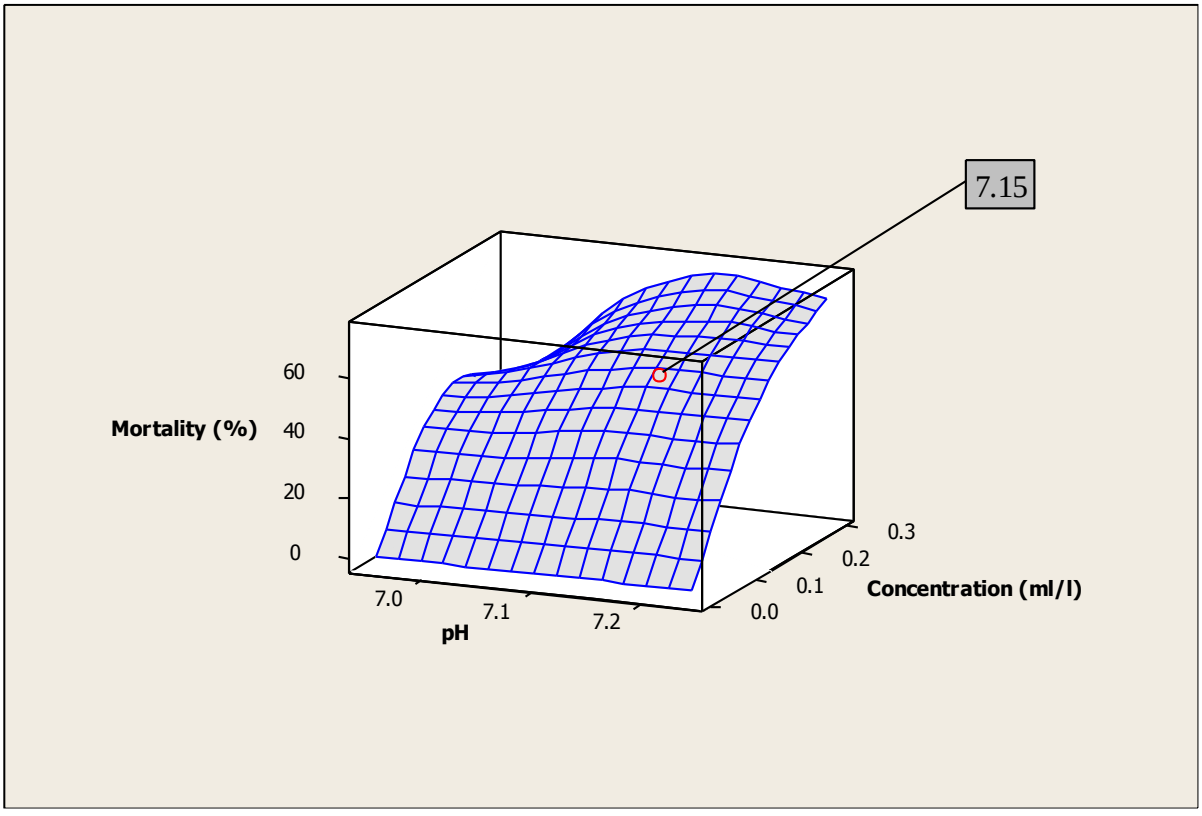

Figure 4. Corresponding pH for LC50 for *C. gariepinus* juveniles exposed to various concentrations of atrazine.

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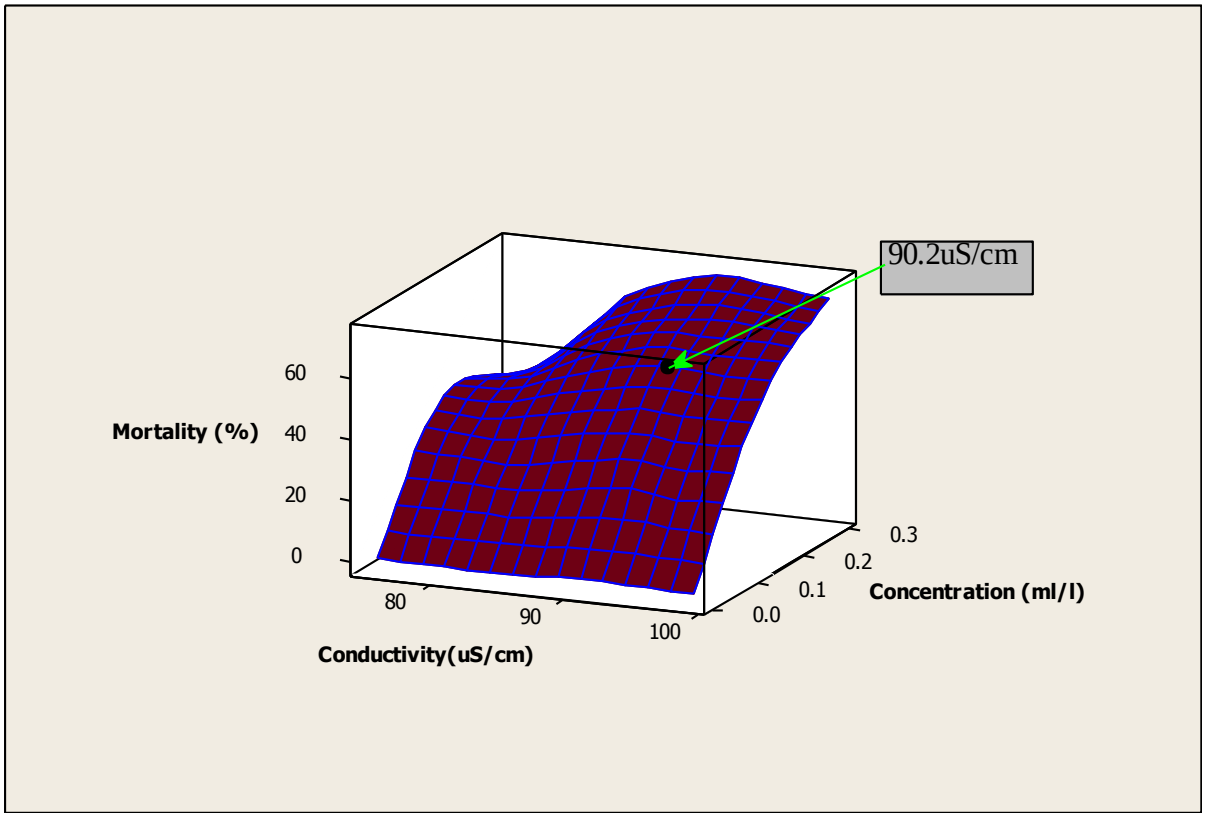

Figure 5. Corresponding conductivity for LC50 for *C. gariepinus* juveniles exposed to various concentrations of atrazine.

Table 3. Enzymatic analysis of the *C. gariepinus* exposed to different concentration of Atrazine (Means±SE)

PARAMETER	0.00	0.10	0.15	0.20	0.25	0.35
AST	8.63±0.25^{f}	17.50±0.51^{e}	23.73±0.61^{d}	34.12±0.61^{c}	49.78±0.70^{b}	58.59±0.50^{a}
LDH	7.66±0.12^{f}	8.46±0.22^{e}	9.38±0.16^{d}	10.60±0.09^{c}	11.61±0.24^{b}	14.54±0.15^{a}
ALT	16.90±0.96^{e}	20.57±0.16^{d}	25.46±0.68^{c}	27.67±0.91^{c}	31.16±0.70^{b}	34.98±0.85^{a}

Means with similar superscripts on the same row are not significantly (P>0.05) different. AST: aspartate aminotransferase, LDH: lactate dehydrogenase, ALT: alanine aminotransferase.

Nigerian (Ita, 1980) are widely cultured in ponds and also occur freely in Natural Freshwater in Africa and other continent . According to Musa and Omoregie, (1999) fish are intimately associated with the aqueous environment, physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish.

The result obtained in this study showed that the behavioural response of the *C. gariepinus* juveniles exposed to atrazine increased with the concentration and time of exposure. The fishes exhibited erratic swimming, air gulping, loss of reflex, colour change and restlessness, mucus secretion, and haemorrhage.

These behaviour alterations were in accordance with the report of Chapadense, Castro, Almeida, & Moron (2009) who evaluated the toxic effects of atrazine on Tambaqui (*Colossoma macropomum*), where abnormal behaviours such as lethargy, loss of equilibrium, increase in the frequency of opercula movements and increase in the thickness of the inferior lips after 48hrs of exposure to atrazine at the range of 20-25 mg/L were observed. There was haemorrhages in the whole body of the fish in the treatment groups, and other signs included loss of reflex, erratic swimming and change in colour are indication of stress which might have led to mortality (Chan, 1982). Similar observation was reported by Radon & Harrel (1990) during exposure of *C. gariepinus* juveniles to formalin. Fish behavioural changes were minimal at low concentration, showing that low concentration of the atrazine could be tolerated by juvenile *C. gariepinus*, thus reduced mortality. The mean mortality of the test fish exposed to various concentrations of atrazine is presented in Figure 1.The present study showed that

fish mortality increased with increase in concentration of toxicant (atrazine). This agreed with the observation of Chan & Lei (1990) who reported that juvenile *C. gariepinus* tolerated differences levels of herbicide. The minimum fish mortality rate was observed in the control where none of the fishes died. The maximum mortality was observed in the treatment 5 (0.30ml/L), where 70% of the fishes were dead. The variabilities in water quality parameters had been reported to have severe effect on the organisms living within the medium especially fishes (Greig, Sear, & Carling 2005). This is because their homeostatic mechanisms are highly dependent on existing conditions in their immediate surrounding parameters (Nussey, Van Vuren, & Preez, 1995). In this study, the pH values (6.95 ±0.17 to 7.24±0.00) observed are in within tolerant range as reported by Witcschi & Ziebell (1979) for tropical fishes. The value of DO was noticed to decrease with increasing level of atrazine. This is similar to the work of Warren (1977) who reported that the introduction of a toxicant into an aquatic system might decrease the dissolved oxygen concentration which will in turn impair respiration thereby leading to asphyxiation. A significant difference observed in conductivity across the treatment might also be as result of varying level of toxicant. However, these changes in water parameters were within the acceptable limits for fish culture according to Boyd (1982). Physiological stress biomarkers can be used as a criterion when defining effect of atrazine on aquatic organism. It serves as indicator for environmental stress on fish (Conn, Christopoulos, & Lindsley, 2009). In the present study, blood biomarkers were analysed for AST, LDH and ALT. The result inferred that, atrazine administration led to increase levels of blood markers (AST, LDH, and ALT). The values recorded for all the enzymes increased across the treatment. However, there were significant ($P<0.05$) differences across the treatment. The significantly higher AST, LDH, and ALT activities in the fish exposed to increasing atrazine across the treatment when compared with control groups could be as a result of leakage of aminotransferase (AT) enzymes from injured liver cells. These results were similar to that of Konstantinova & Russanov, (1999) who studied paraquat induced oxidative stress in rat liver. The increase in the value of the enzymes activity may be as a result of extra-cellular fluid as reported by Akanji, Olagoke, & Oloyede (1993)

Both the AST and ALT are enzymes connected with liver parenchymal cells. They are elevated when there is acute liver impairment (Scola *et al*. 2000; Lin *et al*. 1999). These enzymes also present in red blood cells, heart cells, muscle tissue, pancreas and kidneys. When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST and ALT are released into the bloodstream. Both ALT and AST levels are reliable indicators of liver damage. In short, increase in serum ALT and AST has been reported in conditions involving necrosis of hepatocytes (Macfarlane, Merrison, Dinsdale, & Cohen, 2000), myocardial cells, erythrocyte and skeletal muscle cells (Halworth & Capps, 1993). Alteration in serum/tissue levels of AST, ALT and ALP as recorded in this studies are indications of derangement in cellular activities and hence toxicity, however, the toxicity is mild in the lower concentration compared to the higher concentrations.

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